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CYTOTAXONOMICAL STUDIES ON THE *CALTHA PALUSTRIS* COMPLEX (RANUNCULACEAE) IN POLAND. PRELIMINARY REPORT

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The karyology of representatives of morphologically differentiated taxa within the *Caltha palustris* complex was studied. Examination of plants from ten populations of *C. palustris* subsp. *palustris* from Poland indicated the domination of somatic chromosome numbers 2n = 32 and 56, with 2n = 32 and 56 for var. *palustris*, 2n = 56 for var. *radicans* and 2n = 56 for var. *cornuta*. *C. palustris* subsp. *laeta* from the Tatra Mts. had chromosome number 2n = 62. Mixoploidy was characteristic of the material, with a range of euploid and aneuploid chromosome numbers from 2n = 25 to 2n = 94. Only three plants (from Mrzeżyno, Duninowo and Olkusz) uniformly had 2n = 32. Detailed biometric analyses seem to point to the lack of a simple relation between karyological and morphological variability in representatives of the *C. palustris* complex occurring in Poland.

Key words: *Caltha palustris* L. s.l., cytological variability, chromosome numbers, morphological variability, Poland.

INTRODUCTION

The genus *Caltha* L., with two allopatric subgenera *Caltha* and *Psychrophila* (Gay) Prantl, consists of about 40 species (Ziemmerman, 1965). The Linnaean species *Caltha palustris*, which belongs to the subgenus *Caltha*, is a circumboreal taxon, common in Poland in both the lowlands and the mountains, where it reaches up to the alpine belt. It grows in wet and marshy areas.

The great differentiation of morphological features (habit, shape of leaves and follicles) led to the division of Linnaean species *C. palustris* into independent species and/or subspecific taxa. Schott et al. (1854) distinguished six species within the complex: *C. cornuta*, *C. latifolia*, *C. laeta*, *C. intermedia*, *C. vulgaris* and *C. alpestris*. This classification was later modified several times. On the basis of follicle polymorphism Hegi (1912) distinguished three subspecies within *C. palustris* s.l.: subsp. *genuina*, subsp. *cornuta* (Schott, Nyman et Kotschy) Beck, and subsp. *laeta* (Schott, Nyman et Kotschy) Hegi. Taking into account some extra features such as chromosome number, habitat and phytocoenoses, Ziemmermann (1965) divided the *C. palustris* complex into four subspecies, adding subsp. *minor* (Mill.) Graebner to those of Hegi (1912). In both editions of *Flora Europea* (Tutin, 1964; Akeroyd, 1993) the wide embrace of the Linnaean *C. palustris* is accepted without any infraspecific taxa distinguished, but the enormous morphological and cytological variability of the species is stressed.

The range of variability and the systematics of the *C. palustris* complex in Poland has not been clarified yet. Paczoski (1927) divided it after Schott et al. (1854) into three species (i.e. *C. cor-*

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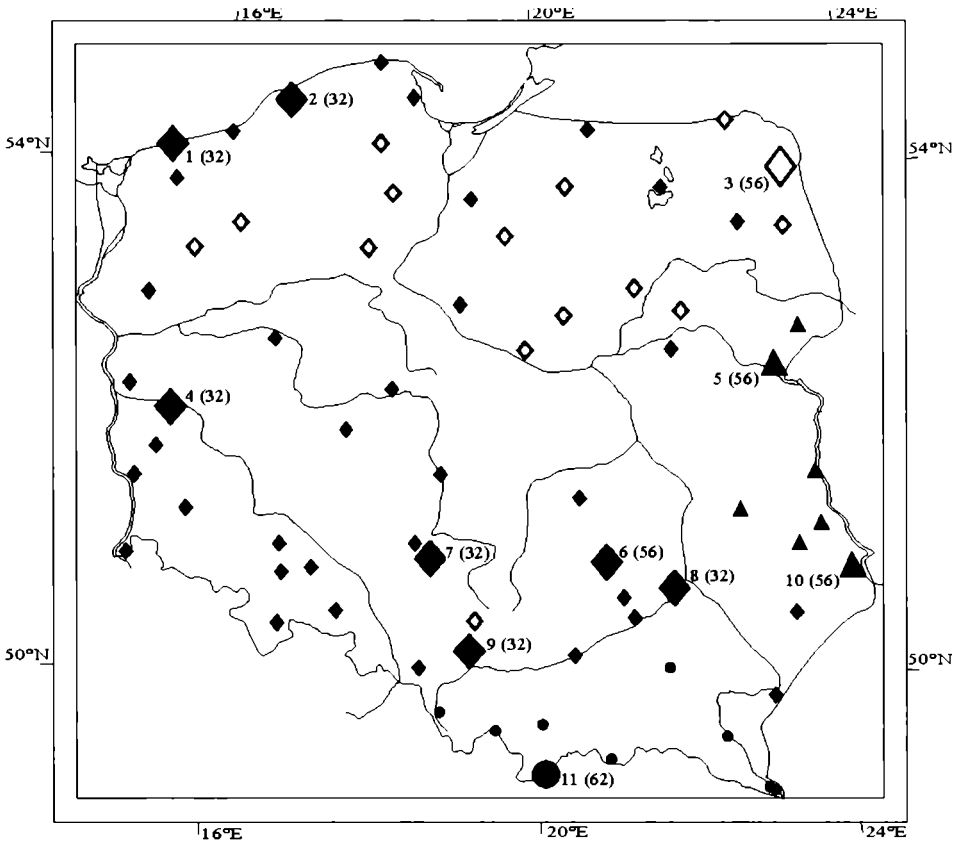


Fig. 1. Distribution of 74 examined samples of *Caltha palustris* s.l. in Poland. ● *C. palustris* subsp. *laeta*; ◆ *C. palustris* subsp. *palustris* var. *palustris*; ◊ *C. palustris* subsp. *palustris* var. *radicans*; ▲ *C. palustris* subsp. *palustris* var. *cornuta*. The specimens from which samples were taken for cytological tests are marked with larger symbols; the same populations are in Table 2; somatic chromosome numbers (2n) in parentheses.

nuta Schott, Nyman et Kotschy; *C. laeta* Schott, Nyman et Kotschy; *C. palustris* L.). Kucowa (1985) distinguished two species within the Linnaean *C. palustris* (*C. palustris* L. s.str. and *C. laeta* Schott, Nyman et Kotschy). She also recognised two subspecies within *C. palustris* – subsp. *palustris* and subsp. *cornuta* (Schott, Nyman et Kotschy) Hegi – and two varieties within *C. laeta*, that is, var. *laeta* and var. *alpestris* (Schott, Nyman et Kotschy) Beck. Recently, Mirek et al. (1995) accepted Ziemmerman's (1965) division of the *C. palustris* complex into three subspecies: subsp. *laeta* (Schott, Nyman et Kotschy) Hegi, subsp. *palustris* and subsp. *cornuta* (Schott, Nyman et Kotschy) Hegi. The division of the complex accepted in the present paper distinguishes three varieties of *C. palustris* L. subsp. *palustris*: var. *palustris*, var. *radicans* (Forster) Beck, var. *cornuta* (Schott, Nyman et Kotschy) Borbás and *C. palustris* subsp. *laeta* (Schott, Nyman et Kotschy) Hegi.

The great morphological variability of *C. palustris* s.l. is accompanied by karyological differentiation. Different euploid and aneuploid chromosome numbers have been noted in ploidy levels ranging from 2n = 2x = 16 to 2n = 11x = 88. The results of hitherto known karyological investigations are presented in Table 1.

The present work tests the applicability of karyological investigations in solving the taxonomic problems that arose during research on the morphological variability of this complex.

MATERIALS AND METHODS

MORPHOLOGY

For morphometric analysis of the *C. palustris* complex, 74 population samples of 25–30 plants each were collected from the whole area of Poland; all the main geographical regions of the country were rep-

TABLE 1. Previous chromosome reports for the *Caltha palustris* complex

Provenience of the material	Chromosome numbers 2n	Author
NORTH AMERICA		
Canada	32, ca 32, 56	Wcisło, 1967; Kootin-Sanwu and Woodell, 1970
U.S.A.	32, 56, 60, ca 64	Wcisło, 1967
EUROPE		
Austria	32, 52, 56, 57, 58, ca 58, 59, ca 59, 60, ca 60, 61, ca 61, 64	Wcisło, 1967; Smit, 1967; Reese, 1954; Kootin-Sanwu and Woodell, 1970
Czech Republic	32, 34, 35, 36, 44, 47, 55, 56, ca 56, 57, 58, 59, 61, 64	Chrtkova and Jarolimova, 1999
Denmark	56	Reese, 1954
France	32, ca 32, 48, 56	Reese, 1954; Smit, 1967; Kootin-Sanwu and Woodell, 1970
Germany	16, 28, 29, 30, 32, ca 32, 55, 56, ca 56, 57, ca 57, 58, ca 58, 59, 60, ca 60, ca 61	Engler, 1936; Reese, 1954; Wcisło, 1967; Smit, 1967; Kootin-Sanwu and Woodell, 1970; Goldblatt, 1981
Great Britain	52, 54, 55, 56, 57, 58, 60, 64, ca 64, 72, ca 80	Löve and Löve, 1948; Reese, 1954; Wcisło, 1967; Kootin-Sanwu and Woodell, 1970
Greece	32	Goldblatt, 1984
Hungary	32	Polya, 1950
Iceland	48, ca 48, 53, ca 54, 55, 56, 48–80	Löve and Löve, 1948, 1956; Reese, 1954
Italy	16, 24, 30, 32, ca 32, 33, 34, 36, 40, 42, 43, 44, 46, 48, ca 48, 50, 52, 56, ca 56, 58, 60, 61, 62, 63, 64, 72	Reese, 1954; Kootin-Sanwu and Woodell, 1970; Leoncini, 1951, 1952
Netherland	32, 56, 57	Reese, 1954; Smit, 1967
Norway	32, 56, ca 56	Reese, 1954; Kootin-Sanwu and Woodell, 1970
Poland	32, 48, 56, 58, 60	Skalińska et al., 1959; Wcisło, 1964, 1967, 1968; Falińska, 1981; Werpachowski, 1994
Portugal	32	Smit, 1967
Slovakia	32, 56	Májovský et al., 1974; Hrušovská-Osuská, 1988
Spain	32, 56	Smit, 1967
Sweden	28, 32, 48, 56, 59	Reese, 1954; Kootin-Sanwu and Woodell, 1970; Wcisło, 1967
Switzerland	60, ca 60	Reese, 1954
Former U.S.S.R.	16, 28, 32, 48, 56, ca 56, 60, 64	Wcisło, 1967; Rostovtseva, 1981; Goldblatt, 1981, 1984, 1988; Lavrenko and Serditov, 1984, 1985; Goldblatt and Johnson, 1990
Yugoslavia	32	Reese, 1954
ASIA		
Arctic Russia, Pamir and Altai, Kamschatka	32, 48, 56, ca 70	Kootin-Sanwu and Woodell, 1970
Japan	60, 88	Goldblatt, 1985; Goldblatt and Johnson, 1990

resented (Fig. 1). This material was analyzed biometrically according to the following five scored qualitative morphological features:

1 – margin of the top of the basal leaf (entire, serrate, dentate-crenate);
2 – margin of lobes of the basal leaf (entire, serrate, crenate, dentate-crenate);
3 – stem morphology (erect, ascending, creeping);
4 – shape of the beak (straight, weakly bent, bent, hooked);

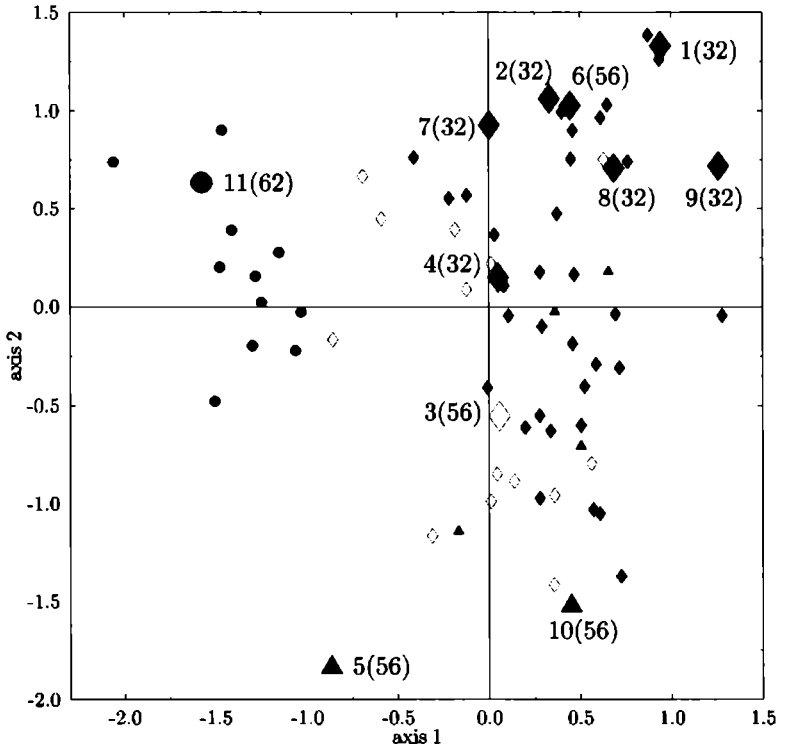


Fig. 2. Scatter diagram of 74 populations of *Caltha palustris* s.l. in Poland, based on correspondence analysis versus axis 1 and axis 2, on the basis of 5 qualitative features (1–5). ● *C. palustris* subsp. *laeta*; ◆ *C. palustris* subsp. *palustris* var. *palustris*; ◊ *C. palustris* subsp. *palustris* var. *radicans*; ▲ *C. palustris* subsp. *palustris* var. *cornuta*. The specimens from which samples were taken for cytological tests are marked with larger symbols; the same numbers of populations are in Table 2; somatic chromosome numbers (2n) in parentheses.

5 – divergence of follicles (follicles straight and pressed, weakly bent, slightly divergent, distinctly bent, divergent, strongly recurved and divergent).

Nine quantitative features were also analyzed:

6 – angle of follicle bending;
7 – depth of sinus of basal leaf;
8 – angle of basal leaf sinus;
9 – ratio of follicle length (measured along the medial curve) to the shortest (straight) distance between the base of the follicle and the top of its beak;
10 – ratio of the difference between dorsal and abdominal edge lengths of the follicle to its length (measured along the medial curve);
11 – ratio of maximal width of basal leaf blade to its length (between apex and base);
12 – ratio of length of basal leaf blade measured along a radius angled 40° from the main axis (connecting the base of the leaf with its apex) to the length of the blade along the main axis;
13 and 14 – ratios as in 12, but with basal leaf blade measured along radii angled 80° and 140° from the main axis, respectively.

Numerical analyses were done based on the biometric data: correspondence analysis (CA) for the qualitative (1–5) features, and discriminant analysis (DA) for the quantitative features (6–14).

The aim was to establish the degree of homogeneity of the complex and the interrelations between the specific morphological groups, and indirectly to determine their variability.

KARYOLOGY

The specimens for karyological studies were collected from eleven natural habitats in Poland (Fig. 1) by the first author. Chromosome numbers were counted in a total of 15 plants representing all

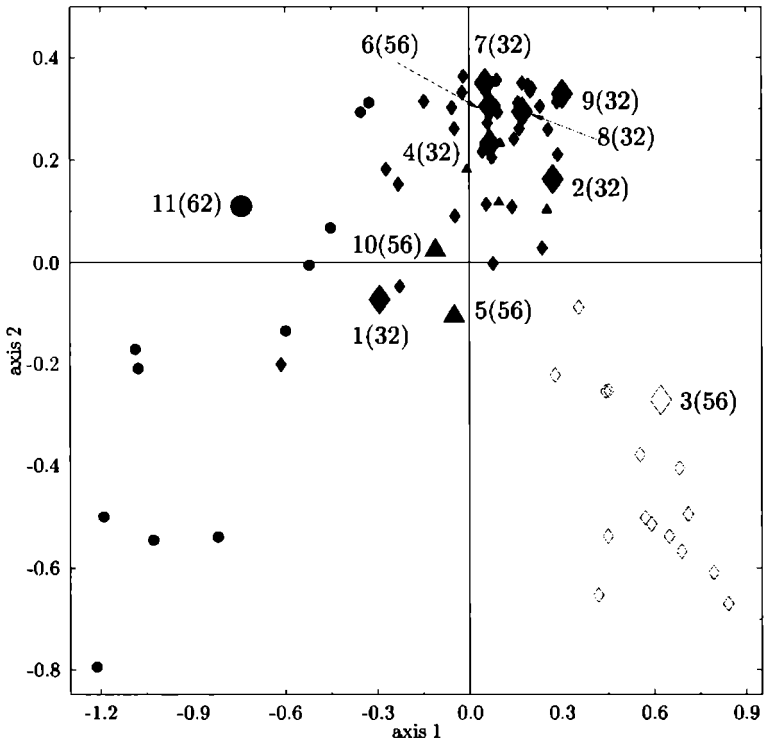


Fig. 3. Scatter diagram of 74 populations of *Caltha palustris* s.l. in Poland based on discriminant analysis versus axis 1 and axis 2, on the basis of 9 quantitative features (6–14). ● *C. palustris* subsp. *laeta*; ◆ *C. palustris* subsp. *palustris* var. *palustris*; ◊ *C. palustris* subsp. *palustris* var. *radicans*; ▲ *C. palustris* subsp. *palustris* var. *cornuta*. The specimens from which samples were taken for cytological tests have been marked with larger symbols; the same numbers of populations are in Table 2; somatic chromosome numbers (2n) in parentheses.

taxa of *C. palustris* s.l. known to occur in the country (Tab. 2).

The plants were placed in vessels with water for rooting. After several days the root tips were excised and pretreated with a saturated water solution of α-bromonaphthalene for 2–4 h and fixed in 1:3 acetic alcohol. The root tips were stained in toto in acetic orcein for two weeks and squashed in 45% acetic acid. The chromosome counts were made in metaphase plates of one or two individuals from each population.

Voucher specimens are deposited in the Herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences in Cracow (KRAM).

RESULTS

MORPHOLOGY

On the basis of CA of 5 qualitative characters (1–5) and DA of 9 quantitative characters (6–14) (Figs. 2, 3), two main groups were distinguished within the *C. palustris* complex. They are classified as two subspecies: subsp. *laeta* and subsp. *palustris*. Within subsp. *palustris*, apart from var. *palustris*, var. *radicans* was distinguished on the basis of the CA results (Fig. 2). The second variety has characteristically developed features as analyzed biometrically, but with procumbent stem roots at the nodes. DA provided grounds for differentiating a group of specimens classified as var. *cornuta* (Fig. 3). This variety has divergent follicles and a large angular divergence between the basal lobes. However, the range of morphological variability within this group largely overlaps that of var. *palustris*.

The lack of distinct divisions within the complex, and the occurrence of transitional forms not discussed in the present paper, points to the gradual character of variability within the *C. palustris* complex in Poland (Figs. 2, 3). The morphotypes distinguished within the *C. palustris* complex are assigned a low rank because of the lack of discontinuity in variability between these groups (Figs. 2, 3).

TABLE 2. Results of karyological studies of the *Caltha palustris* complex in Poland

Taxon	Locality (No)	N. lat.	E. long.	2n
C. palustris L. subsp. *palustris* var. *Palustris*	Mrzeżyno (1)	54°05'	15°17'	32
	Duninowo (2)	54°32'	16°49'	32
	Radnica (4)	52°05'	15°15'	32
	Suchedniów (6)	51°03'	20°50'	56
	Lipie (7)	51°01'	18°48'	32
	Sandomierz (8)	50°42'	21°45'	32
	Olkusz (9)	50°17'	19°04'	32
C. palustris L. subsp. *palustris* var. *radicans*	Frącki (3)	54°00'	23°20'	56
C. palustris L. subsp. *palustris* var. *cornuta*	Zalesie-Moszczona Pańska (5)	52°27'	23°01'	56
	Hrubieszów (10)	50°48'	23°53'	56
C. palustris L. subsp. *laeta*	Dolina 5 Stawów Polskich – Tatra Mts (11)	49°13'	20°02'	62

KARYOLOGY

Great variability in somatic chromosome number was found in the plants examined (2n from 25 to 94). The plants from Mrzeżyno, Duninowo and Olkusz, with a uniform chromosome number (2n = 4x = 32) in all root-tip metaphases, were exceptional. In most cases the chromosome number varied within individuals; different cells in their root-tips had different euploid and aneuploid chromosome numbers (mixoploidy). In each mixoploid plant, the most frequently observed chromosome number (2n = 32, 2n = 56, or 2n = 62) was treated as the main chromosome number, following Reese's rule (1954). These chromosome numbers are presented in Table 2.

In the eleven populations from Poland, somatic chromosome numbers 2n = 32, 2n = 56 and, in one case from the Tatra Mts., 2n = 62 were found (Fig. 4). More than one chromosome number, both euploid and aneuploid, were observed within one plant or even within one root: *C. palustris* subsp. *palustris* var. *radicans* (from Frącki) – 2n = 56, 64; *C. palustris* subsp. *palustris* var. *palustris* (from Radnica) – 2n = 25, 32, 60; *C. palustris* subsp. *palustris* var. *cornuta* (from Zalesie-Moszczona Pańska) – 2n = 42, 47, 56, 94; *C. palustris* subsp. *palustris* var. *palustris* (from Suchedniów) – 2n = 35, 49, 52, 56; *C. palustris* subsp. *palustris* var. *palustris* (from Lipie) – 2n = 28, 29, 32, 40, 56; *C. palustris* subsp. *palustris* var. *palustris* (from Sandomierz) – 2n = 30, 32, 35, 40, 42, 52, 53; *C. palustris* subsp. *palustris* var. *cornuta* (from Hrubieszów) – 2n = 39, 41, 48, 56, 64, 72; and *C. palustris* subsp. *laeta* (from Dolina 5 Stawów Polskich – Tatra Mts.) – 2n = 53, 57, 58, 59, 60, 61, 62.

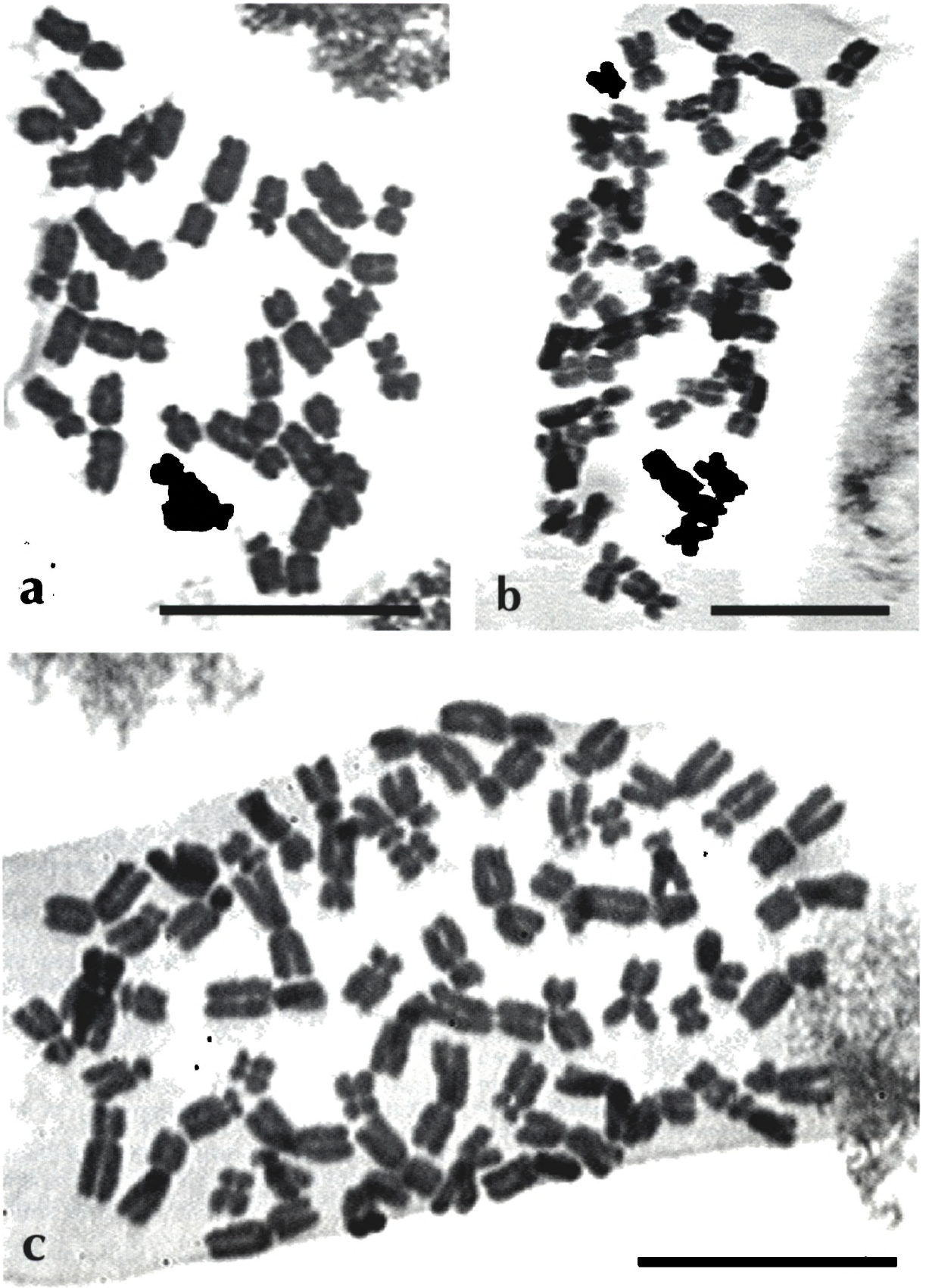
The *C. palustris* metaphase plates show differentiation of chromosome morphology. The largest chromosome of the complement is about three times the length of the smallest one.

DISCUSSION

None of the morphological or karyological research done so far has succeeded in dividing the *C. palustris* complex in Poland into unambiguous taxa (Wcisło, 1967; Falińska, 1974, 1976, 1981).

Our morphological studies confirmed the existence of a quite uniform group of plants from mountain habitats, classified as *C. palustris* L. subsp. *laeta* (Figs. 2, 3). The morphotype is characterized by a rounded leaf blade, a serrate or dentate-crenate basal leaf margin, and straight and pressed follicles leading to a short and straight beak. Wcisło (1967) established chromosome number 2n = 56 for this taxon from Poland. These results do not correspond exactly with Chrtkova and Jarolimova's (1999) recent data from the Czech Republic and Slovakia. They found two somatic chromosome numbers in *C. palustris* subsp. *laeta*: 2n = 32 (from hilly habitats) and 2n = 56 (from mountain habitats). Wcisło (1967) investigated several populations of subspecies from Bulgaria and determined one chromosome number, 2n = 32. In the present work, chromosome number 2n = 62 was found in this taxon in a population originating from the Tatra Mts. (Tab. 2). This number has not been reported in this subspecies before, and needs to be confirmed. This chro-

Fig. 4a–c. Metaphase plates of *Caltha palustris* s.l. (**a**) *C. palustris* subsp. *palustris* var. *palustris* (2n = 32; Duninowo), (**b**) *C. palustris* subsp. *palustris* var. *cornuta* (2n = 56; Hrubieszów), (**c**) *C. palustris* subsp. *laeta* (2n = 62; Tatra Mts). Bar = 10 μm.


a
b
c

mosome number has been reported from Italy for *C. palustris* subsp. *palustris* (Leoncini, 1952).

The data shows that morphologically subsp. *laeta* is a relatively homogenous taxon, and karyologically it is a heterogeneous one. Both 2n = 32 and 2n = 56 cytotypes have been reported for this taxon in Europe (Satczek in Skalińska et al, 1959; Wcisło, 1967; Chrtkova and Jarolimova, 1999).

Caltha palustris subsp. *palustris* is a much more variable taxon morphologically, as is confirmed by research from other parts of Europe (Woodell and Kootin-Sanwu, 1971; Smit, 1967). Our investigations showed the morphotype in Poland to be characterized by an erect stem, weak or distinct and divergent follicles, 30–70° angular divergence between basal lobes, and distinctly crenate to deeply dentate basal leaf margins. Biometric analysis distinguished three varieties within this subspecies: var. *palustris*, var. *cornuta* and var. *radicans*.

Varietas *palustris* is characterized by an erect stem, slightly divergent follicles, and a 40–60° angle sinus of the basal leaves. Varietas *radicans* is the most distinctive of the three. It is characterized by an ascending and creeping stem, mentioned also by Smit (1967, 1973). Less distinctive is var. *cornuta*, characterized by its strongly recurved and divergent follicles, kidney-shaped basal leaves, large-angle sinus of the basal leaves, and entire or dentate-crenate basal leaf margin. Between the varieties there are many transitional forms, whose status is not discussed here.

Caltha palustris subsp. *palustris* is equally variable in terms of karyology. Populations of subsp. *palustris* from Poland were characterized by the occurrence of one specific cytotype, 2n = 56, except for one population with 2n = 60 (Wcisło, 1967). The specimens from the Czech Republic and Slovakia representing this taxon examined by Chrtkova and Jarolimova (1999) had chromosome number 2n = 32, and there are individual reports of 2n = 34, 2n = 35 and 2n = 36.

We also found cytological differentiation in the specimens of subsp. *palustris*, where numbers 2n = 32 and 2n = 56 were counted. In var. *palustris*, chromosome number 2n = 32 was counted in representatives of six populations, and 2n = 56 in one population. In var. *cornuta* and var. *radicans* 2n = 56 was found (Tab. 2).

Just as in subsp. *laeta*, on the basis of the data it is hard to decide on a number specific to subsp. palustris. As in the case of varieties distinguished morphologically, there is no specific cytotype (Tab. 2).

So far the research on *C. palustris* s.l. has shown great karyological variability within this complex. Different euploid and aneuploid chromosome numbers have been noted at ploidy levels from 2n = 2x = 16 to 2n = 11x = 88 (Tab. 1). Tetra- and heptaploid cytotypes (2n = 32, 56) are most common in Europe, but di-, tri-, hexa-, octo- and nanoploid (2n = 16, 24, 48, 64, 72) individuals have also been noted, together with aneuploids from 2n = 38 to 2n = 70 (Kootin-Sanwu and Woodell, 1970; Tab. 1).

Previous authors have reported different chromosome numbers within one plant (Leoncini, 1951, 1952; Reese, 1954; Kootin-Sanwu and Woodell, 1970). We also observed several numbers of chromosomes in one individual. Reese (1954) suggested that the number most often repeated can be taken as the main somatic number for plants with different chromosome numbers within their root tips.

Satczek (in Skalińska et al., 1959) and Wcisło (1967, 1968) found individuals with 2n = 32, 56 to be dominant in Poland, confirming observations from other parts of Europe (Smit, 1973). They also occasionally found plants with chromosome numbers 2n = 48, 52, 58 and 60.

The results of detailed biometric analyses seem to rule out a simple relation between karyological and morphological variability in representatives of the *C. palustris* complex (also mentioned by Reese, 1954). In Poland, cytological variability is so great in the morphologically differentiated groups that taxa cannot be distinguished on the basis of their chromosome numbers. We can only state that, as in other parts of Europe (Smit, 1973), the two most common cytotypes in Poland are 2n = 32 and 2n = 56. The difficulty in interpreting karyological differentiation within *C. palustris* s.l. is due to mixoploidy (Reese, 1954).

The great karyological variability (Tab. 1) and relatively weak differences between the groups, together with the presence of intermediate forms and the lack of genetic barriers (Wcisło, 1964, 1968), presumably means that *C. palustris* is a "young evolutionary taxon" (Hoffman, 1999).

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